

REMARKS

I. Status Summary

Claims 1-6 and 8-10 are pending in the present application. Claim 10 has been withdrawn for being drawn to a non-elected invention. Claims 1-6, 8 and 9 presently stand rejected. Claim 7 is cancelled.

Claims 1-6 and 8 have been amended. Support for the claim amendments can be found throughout the application, in particular in the original claims. No new matter has been added.

New claim 11 has been added. Support for claim 11 can be found in at least original claim 9 and Example 2.

II. Responses to the Rejections under 35 U.S.C. §112, first paragraph

The Patent Office rejects claims 1-6, 8 and 9 under 35 U.S.C. §112, first paragraph, for lack of enablement. The Patent Office contends that there is insufficient support for differentiating mesenchymal stem cells (MSC) into any hormone-producing cells, though the Patent Office acknowledges that there is support for cells that produce progestin and androgen. The Patent Office also acknowledges that there is support for differentiating mesenchymal stem cells using SF-1 and cAMP together, but contends that support is lacking for using the SF-1 protein to induce differentiation in culture or in the absence of cAMP or for inducing differentiation by transplanting the stem cells *in vivo*. The Patent Office cites Val et al., Nuclear Receptor, 1: 1-23 (2003), hereinafter "Val," in support of the contention that the effects of SF-1 on target genes are unpredictable.

Applicants respectfully traverse this rejection and point out that neither Val nor Crawford discuss differentiating mesenchymal stem cells.

The Patent Office contends that there is insufficient support for differentiating MSC into any hormone-producing cells. Applicants direct the attention of the Patent Office to the Example section of the Specification.

Example 1 discloses that MSC transfected with a vector to express SF-1 protein contained fat droplets. The SF-1 expressing cells also expressed p450scc (cholesterol side chain cleavage enzyme), an enzyme associated with the conversion of cholesterol

to pregnenolone. Pregnenolone is a steroid hormone involved in steroidogenesis of progesterone, mineralocorticoids, glucocorticoids, androgens, and estrogens. Paragraph [0040] discloses examples of steroid hormones synthesized from cholesterol including progestin, androgen, estrogen, glucocorticoid and mineralcorticoid.

Example 2 discloses that MSC transfected with SF-1 expressed p450scc, HSD3b1 (3- β hydroxysteroid dehydrogenase), and p450c17 (17-alpha-hydroxylase/17,20 lyase). Progesterone, androgen, and androstenedione were also produced. The enzyme, 3- β HSD, catalyzes the synthesis of steroid hormones, such as progesterone and androstenedione. Androstenedione is an intermediate in the steroid hormone pathways of the androgen, testosterone, and the estrogens, estrone and estradiol. The enzyme p450c17 acts on steroid hormones pregnenolone and progesterone.

In Example 3, MSC were differentiated into steroid hormone producing cells which express p450scc, StAR (steroidogenic acute regulatory protein), 3 β -HSD, and the glucocorticoid producing enzymes p450 c21 (steroid 21-hydroxylase) and p450 11b1 (steroid 11-beta- hydroxylase). StAR regulates cholesterol transfer in mitochondria which is a rate limiting step in steroid production.

In Example 4, MSC cells were differentiated into Leydig cells, which produce testosterone and other androgens. The differentiated cells also expressed p450scc.

In Example 5, MSC were differentiated into steroid hormone-producing cells; cells which expressed p450scc, 3 β -HSD, HSD3b1, and HSD3b6.

In view of the above remarks, applicants respectfully submit that Examples 1-5 provide support for claim 1, as presently presented. The disclosure of the instant application is not limited to progestin and androgen as the Patent Office contends. Applicants respectfully submit that there is enabling support for MSC, differentiated by stimulation with SF-1, which produce many steroid hormones, their precursors, intermediates, and regulators.

However, to further the prosecution, and without acquiescing to the Patent Office's contention, applicants have amended claim 1 to include the phrase "wherein the hormones produced are selected from the group consisting of progestin, androgen, estrogen, glucocorticoid, and mineralcorticoid." Support for new claim 1 can be found in original claim 9. Claim 9 has been cancelled. It is respectfully submitted that claim

1 is now in proper condition for allowance. Accordingly, applicants request that the rejection of claim 1 and its dependent claims be withdrawn.

Differentiation in the Absence of cAMP

The Patent Office asserts that there is a lack of support for inducing differentiation in MSC in the absence of cAMP. The Patent Office cites Crawford et al., Mol. Cell. Biol., 17: 3997-4006 (1997), hereinafter "Crawford," as evidence that cAMP is required to differentiate MSC. Applicants submit that there are significant technical differences between the present subject matter and that of Crawford.

Applicants respectfully request that the Patent Office direct its attention to the "Materials and Methods" section Crawford, page 3998, left column, lines 20-21; and page 4000, left column, lines 5 to 4 from the bottom. Crawford used embryonic stem (ES) cells in studies. As described therein, to produce steroid hormone in ES cells stimulated by SF-1, it was necessary to add an intermediate of steroid synthesis, 20 α -hydroxycholesterol, as a substrate. In Crawford, steroids are synthesized only in the presence of an intermediate of steroid synthesis. The intermediate is necessary because stimulation by SF-1 is not sufficient to induce the gene expression necessary for the early stage of steroid synthesis. Thus without the addition of the intermediate, steroids are not produced in ES cells. Addition of the intermediate along with SF-1 induces the gene expression necessary for the later stage of steroid synthesis wherein steroids can then be produced.

As can be seen in the present subject matter, in contrast to Crawford, stimulation with SF-1 provides all necessary gene expression for steroid synthesis in MSC. MSC are differentiated into steroid producing cells, as described in the description and Examples of the present patent application, to produce steroids. Crawford discusses experiments in which intermediates of steroid synthesis are added to ES cells. In the presently disclosed subject matter, it is not necessary to add an intermediate as in Crawford. Expression of all necessary genes for the synthesis of steroids can be induced by SF-1 in mesenchymal stem cells.

Regarding the enablement for differentiating MSC in the absence of cAMP, applicants direct the attention of the Patent Office to Examples 2 and 4 of the present

application. Experiments in Example 2 compare the results of SF-1⁺ and SF-1⁻ cells grown with cAMP. Those cells containing SF-1 and cAMP expressed p450scc. Cells containing only cAMP did not express p450scc. The results indicate that cAMP alone is not enough to differentiate MSC into steroid hormone-producing cells. In Example 4, mesenchymal stem cells described therein are injected in rat testes wherein they differentiate into Leydig cells, without the addition of cAMP. Applicants respectfully submit that the specification is enabling for the differentiation of MSC into steroid-hormone-producing cells by stimulation with SF-1 without adding cAMP.

However, to further the prosecution, and without acquiescing to the Patent Office's contention, applicants have amended claim 1 to include the phrases, stimulating the MSC "in the presence of cAMP," and "wherein the hormones produced are selected from progestin, androgen, estrogen, glucocorticoid, and mineralcorticoid." Support for new claim 1 can be found in original claims 2 and 9 and on page 5, lines 21-24, where it is disclosed that cAMP may exist in the cell and can be utilized. Claims 2 and 9 have been cancelled. It is respectfully submitted that claim 1 is now in proper condition for allowance.

Accordingly, withdrawal of the rejection over claim 1 and its dependent claims is respectfully requested.

Differentiation in culture, in vitro

Regarding claims 5 and 8 and enablement for differentiating MSC in culture and *in vitro*, applicants direct the Patent Office to Examples 2, 3, and 5. In Example 2, MSC were differentiated in culture into cells that expressed the steroid hormone producing enzymes: p450scc, p450c17, and HSD3b1. See also Figures 4 and 5.

In Example 3, human bone marrow MSC were differentiated in culture medium into steroid hormone producing cells which express p450scc, StAR, 3 β -HSD, p450 c21, and p450 11b1.

In Example 5, mouse MSC were differentiated *in vitro*, in medium into steroid hormone-producing cells, cells which expressed p450scc, 3 β -HSD, HSD3b1, and HSD3b6.

Claim 5 has been amended to insert a space where two words ran together. No new matter has been added.

In view of the enabling disclosure outlined above, applicants submit that claims 5 and 8 are in condition for allowance and that the present subject matter is enabling for the differentiation of MSC *in vitro*. Accordingly, applicants respectfully request that the rejection of claims 5 and 8 be withdrawn.

Differentiation by Transplanting MSC in vivo

Regarding claim 6 and enablement for differentiating MSC by transplanting the cells *in vivo* or into a mammalian reproductive organ, applicants direct the Patent Office to Example 4 of the present application. As described therein, Green rat MSC were transplanted into a mammalian reproductive organ, the testis of a Sprague Dawley rat. Once transplanted, the MSC differentiated into Leydig cells, cells which produce testosterone and other androgens. Applicants respectfully submit that Example 4 provides enablement for the *in vivo* differentiation of MSC and for claim 6 and that undue experimentation is not required to perform the methods in Example 4.

Claim 6 has been amended to more clearly recite the present subject matter. Support for new claim 6 can be found in Examples 4 and 5 and in original claim 6. Example 4 discloses that MSC transplanted into rat testes (a mammalian reproductive organ) can differentiate into Leydig cells, which produce steroid hormones. No new

matter has been added. It is respectfully submitted that claim 6 is now in proper condition for allowance. Accordingly, withdrawal of the rejection over claim 6 is respectfully requested.

III. Responses to the Rejections under 35 U.S.C. §112, second paragraph

The Patent Office also rejects Claims 1-6, 8 and 9 under 35 U.S.C. §112, second paragraph, upon the contention that the phrase, "transcription factor (SF-1)" is indefinite. The Patent Office prefers the phrase, "steroidogenic factor (SF-1)."

Applicants respectfully traverse the rejection. Applicants believe that SF-1 is rightfully called a transcription factor. It is recognized in the art that SF-1 is a transcription factor. One need look no further than to references cited by the Patent Office. "SF-1 is an orphan member of the nuclear receptor superfamily of transcription factors" (Crawford, p. 4002). "[T]hese surprising results shed light on transcription factors other than SF-1"(Val, p. 9 of 23). Furthermore, SF-1 is called a "transcriptional factor" throughout the specification, including for example in the abstract; claim 1; and at page 2, lines 1 and 16. Applicants submit that SF-1 is a transcription factor and that the phrase "transcriptional factor (SF-1)" is well supported in the specification.

Without acquiescing to the contentions of the Patent Office, and to further the prosecution, applicants' herein amend claim 1 to more clearly recite the present subject matter. The parentheses around "SF-1" have been removed and a comma has been inserted following the phrase, "transcriptional factor." No new matter has been added. It is respectfully submitted that claim 1 is now in proper condition for allowance. Accordingly, withdrawal of this rejection over claim 1 is respectfully requested.

CONCLUSIONS

In light of the above amendments and remarks, it is respectfully submitted that the claims 1-6, 8 and 9 are now in proper condition for allowance, and an early notice to such effect is earnestly solicited.

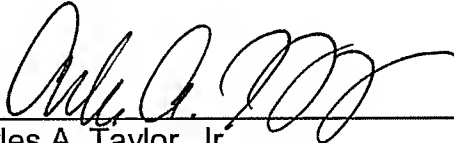
If any small matter should remain outstanding after the Patent Examiner has had an opportunity to review the above Remarks, the Patent Examiner is respectfully requested to telephone the undersigned patent attorney in order to resolve these matters and avoid the issuance of another Official Action.

DEPOSIT ACCOUNT

The Commissioner is hereby authorized to charge any deficiency in payment or credit any overpayment of fees in connection with the filing of this correspondence to Deposit Account No. 50-0426.

Respectfully submitted,
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Date: August 18, 2009

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